Guideline Series 84: MUTAGENICITY

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DATA EVALUATION REPORT

CHEMICAL: CGA-154281 Technical

Tox. Chem. No.: 2980

EPA File Symbol: 7E03489

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER: 407321-04

SYNONYMS/CAS No.:

SPONSOR: Ciba-Geigy Corporation, Greensboro, NC

TESTING FACILITY: Ciba-Geigy Limited, Genetic Toxicology Laboratories, Basal, Switzerland

TITLE OF REPORT: Salmonella/Mammalian-Microsome Mutagenicity Test (Confirmatory gene mutation study with CGA-154281 Tech. in Ames test, which was designed by the Toxicology Branch; See TB MEMO 3/14/88 John Chen)

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STUDY NUMBER(S): 881321

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CONCLUSION(S) - Executive Summary:

CGA-154281 technical (Batch No. FL870211) was nonmutagenic to TA98, TA1537 and TA1538 strains of Salmonella typhimurium in the confirmatory tests with or without metabolic activation at the concentrations tested (DMSO used as solvent).

Concentrations tested: 1st test: 1000, 2000, 3000, 4000, 5000 and 8000 ug/plate; 2nd test: 250, 500, 1000, 2000, 3000 and 4000 ug/plate

Study: Acceptable/Non-acceptable (circle one)
X

PC 211509

SALMONELLA

A. MATERIALS

1. Test Material: Name: CGA-154281 Technical
Description (e.g. technical, nature, color, stability):

Batch #: FL870211 Purity: 95.4%
Contaminants: if reported, list in CBI appendix
Solvent used: DMSO
Other comments:

2. Control Materials:
Negative: DMSO
Solvent/final concentration:
Positive: Non-activation:
Sodium azide _____ ug/plate TA100, TA1535
2-Nitrofluorene 5 & 10 ug/plate ~~TA98~~ TA1538
9-Aminoacridine 50 & 100 ug/plate TA97, TA1537
Other (list):
Daunorubicin-HCl 5 & 10 ug/plate TA98
Activation:
2-Aminoanthracene (2-anthramine) 5 ug/plate
usually all strains
Other (list):

3. Activation: S9 derived from Tif:RAIf(SPF) rats
X Aroclor 1254 X induced X rat ___ liver
___ phenobarbital ___ non-induced ___ mouse ___ lung
___ none ___ hamster ___ other
___ other ___ other
If other, describe below
Describe S9 mix composition (if purchased, give details):

The S9 activation mixture contained 0.3 ml of S9 and 0.7 ml of a solution of cofactor described by Ames et al. (Mutation Res. 31: 347-364, 1975).

4. Test organisms: S. typhimurium strains
___ TA97 X TA98 ___ TA100 ___ TA102 ___ TA104
___ TA1535 X TA1537 X TA1538 ; list any others:
Properly maintained? Y / N (circle one)
Checked for appropriate genetic markers (rfa mutation, R factor)? Y / N (circle one)
The confirmatory study was designed against frameshift strains only

5. Test compound concentrations used:
Non-activated conditions: 1st test: 1000, 2000, 3000, 4000, 5000 and 8000 ug/plate; 2nd test: 250, 500, 1000, 2000, 3000 and 4000 ug/plate.
Activated conditions : 1st test: 1000, 2000, 3000, 4000, 5000 and 8000 ug/plate; 2nd test: 250, 500, 1000, 2000, 3000 and 4000 ug/plate.

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B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - _X_ standard plate test
   - ___ pre-incubation (___ minutes)
   - ___ "Prival" modification (i.e. azo reduction method)
   - ___ spot test
   - ___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if appropriate; e.g. include mediums used, incubation times, assay evaluation):

The tests were carried out in accordance with the method described by Ames et al. (Mutation Res. 31: 347-364, 1975), the OECD guideline 471 (with the exception of statistical analysis) and SOP No. 305001, Ciba-Geigy Ltd., Genetic Toxicology Laboratories. The experimental procedure used in this study is attached.

2. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g. cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):

Based on the results obtained from the two previously accepted tests (Test Nos. 860840 and 86076), CGA-154281 tech. demonstrated statistically significant mutagenic response against TA98, TA1537 and TA1538 strains of S. typhimurium at the dose range from 1000 to 8000 ug/plate. These positive responses against the frameshift strains of S. typhimurium (TA98, TA1537 and TA1538) at 1000, 2000, 4000, 5000 and 8000 ug/plate of CGA-154281 tech. were requested to be repeated in order to determine their reproducibilities as previously recommended by the Toxicology Branch (See TB MEMO 3/14/88 John Chen). Therefore, preliminary cytotoxicity assay for this confirmatory study with CGA-154281 technical is unnecessary.

3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

The mutagenicity of CGA-154281 technical (Batch No. FL870211) in the Salmonella/mammalian activation gene mutation assay was evaluated using three frameshift strains of histidine dependent auxotrophic mutants of S. typhimurium (TA98, TA1537 and TA1538) at the concentrations previously recommended either in the presence or absence of metabolic activation. Results for the non-activated mutation assays show that counts of revertant colonies for each tester strain treated with CGA-154281 tech. were not different than the corresponding controls at the concentrations tested (i.e., 1st test: 1000, 2000, 3000, 4000, 5000 and 8000 ug/plate in Tables 1 and 3; 2nd test: 250, 500, 1000, 2000, 3000, and 4000 ug/plate in Tables 5 and 7). Results for the activated mutation assays show that counts of revertant colonies for each tester strains treated with ~~with~~ CGA-154281 tech. were also not different than the corresponding DMSO-treated controls at the concentrations tested (i.e., 1st test: 1000, 2000, 3000, 4000, 5000 and 8000 ug/plate in Tables 2 and 4; 2nd test: 250, 500, 1000, 2000, 3000 and 4000 ug/plate in Tables 6 and 8). The strain specific control compounds (Daunorubicin-HCl, 9-aminoacridine-HCl, and 2-nitrofluorene) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given the positive responses as expected (Tables 1, 2, 3, 4, 5, 6, 7 and 8 attached).

4. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

(A) The spontaneous revertant colonies for each of the three tester strains of Salmonella typhimurium are found within the normal ranges of revertant colonies recommended by the Salmonella/mammalin-microsome mutagenicity test (Ames et al., Mutation Res. 31: 347-364, 1975).

(B) The strain specific control compound (Daunorubicin-HCl, 9-amino-acridine-HCl, and 2-nitrofluorene) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given significant positive responses over the corresponding negative (DMSO) control values (See results given in Tables 1, 2, 5 and 6). These positive control values demonstrated the sensitivity of the assay system with or without metabolic activation.

(C) The test material, CGA-154281 tech. (Batch No. FL870211), in solution was analyzed to confirm the intended concentrations to be used in the mutagenicity tests (i.e., The content of CGA-154281 tech. in the 2500 and 10000 ug/ml samples of FL870211 was found to be 92% to 95% of the nominal concentrations, respectively; Annex No. 1 attached).

(D) No statistically significant increases (less than 2-fold) in the number of revertant colonies for any of the three tester strains were observed following exposure to the test material (250 through 8000 ug/plate) either in the presence or absence of metabolic activation (See results given in Tables 1, 2, 5 and 6).

(E) Under the test conditions reported, the assay was conducted in a manner to generate valid results. Therefore, the test compound, CGA-154281 technical (Batch No. FL870211) dissolved in DMSO, was not mutagenic against TA98, TA1537 and TA1537 strains of S. typhimurium in the Ames Salmonella/mammalian-microsome mutagenicity test either with or without metabolic activation at the concentrations tested (i.e., 250 through 8000 ug/plate). This confirmatory study is considered adequate and acceptable.

5. Was test performed under GLPs (is a quality assurance statement present)? Y / N (circle one)

6. CBI appendix attached Y / N (circle one)

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